IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	
	)	
Kreutzer *et al.*	)	
	)	**Examiner:**
Serial No.: 09/889,802	)	
(US Nat'l Phase of PCT/DE00/00244	)	
Int'l Pub. No. WO 00/44895)	)	
	)	**Group Art Unit: 1637**
Int'l Filing Date: January 29, 2000	)	
	)	**Confirmation No.:**
For: Method and Medicament for Inhibiting	)	
the Expression of a Defined Gene	)	

CERTIFICATE OF SERVICE UNDER 37 CFR 1.248

Assistant Commissioner for Patents
Washington, D.C. 20231

ATTENTION: John Doll
Director
Technology Center 1600

The undersigned hereby confirms that in accordance with 37 C.F.R. § 1.291, a true copy of the protest filed with respect to this application (with attachments) is being served upon the person believed to be the attorney of record, Kathleen Williams, and upon the applicants/inventors as recited in the PCT application, Roland Kreutzer and Stephan Limmer, and upon the party believed to be the Assignee, Alnylam Pharmaceuticals, in accordance with 37 C.F.R. § 1.248, by courier with return receipt requested, on the date set forth below.

Date: Mar 4, 2004

By: Eric Schellin

Eric Schellin

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PROTEST UNDER 37 CFR 1.291(a)

Assistant Commissioner for Patents
Washington, D.C. 20231,

ATTENTION: John Doll
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Introduction

The protestor requests that the arguments and prior art presented in this protest be considered during examination of this application, which is the U.S. National Phase of PCT/DE00/00244 (an English language copy of this international application as filed in the national phase with the Canadian patent office is attached hereto as Exhibit 1; all references to the specification of the present application are to this document).

The undersigned, a third party to this patent application, protests any allowance of claims reciting a double stranded RNA ("dsRNA") construct comprising not more than 49 base pairs in which at least one strand is complementary to a portion of a gene, including claims to compositions of matter and methods of manufacture and use, except as otherwise indicated below. The application asserts such constructs are useful to inhibit expression of the protein encoded by the gene.

The arguments and prior art submitted herewith demonstrate that the application has no written description support for other than a very limited set of genera and a single species and fails to

enable the full scope of the disclosed genera. In particular, except for a single chemically linked, duplex-forming construct of 21 specified base pairs, the application fails to provide written description support for dsRNA other than genera of "not more than 49" bp and "15-49" bp. The art shows, however, that such dsRNA of fewer than 19 bp and in the range of 29-36 bp are ineffective at inhibiting protein expression. Furthermore, there is prior art that renders these genera anticipated and/or obvious.

The specification provides written description support for only a limited set of genera and a single species

The specification of USSN 09/889,802 (the '802 application) does not satisfy the written description requirement of 35 U.S.C. §112, 1st paragraph, because the scope of the disclosure, while facially broad, in fact does not disclose the invention with sufficient particularity to indicate to one of ordinary skill in the art that the inventors were in possession of the invention throughout its broadest scope.

The specification discloses broadly dsRNA molecules having "not more than 49" and "15-49" base pairs (bp). However, the specification actually discloses only certain species, not all of which fall within this recited scope. To illustrate, Example 1 describes production of an approximately 340 bp dsRNA outside the scope of the generic disclosure of dsRNA molecules 15-49 bp in length. This species was shown to inhibit transcription in an *in vitro* model system. The explicit disclosure of Example 1 is limited to dsRNA 340 bp in length.

Further, Example 2 describes transcription inhibition of an exogenous yellow fluorescent protein (YFP) gene in the murine fibroblast cell line NIH3T3 using a 21 bp dsRNA molecule derived from a 315 bp portion of the YFP gene. However, the specification contains no disclosure relating to how or why this particular 21 bp fragment of the 315 bp region of the YFP gene was chosen as a dsRNA. Moreover, the strands of this dsRNA were linked to an organic (C18) linker, *i.e.*, the 3' end of one strand was covalently linked to the 5' end of the other, complementary strand. The application also explicitly recites that the organic linker is a necessary feature of the smaller dsRNA molecules, stating "[t]his result demonstrates that even shorter dsRNAs can be used for specifically inhibiting gene expression in mammals when the double strands are stabilized by chemically linking the single strands." P. 19, ll. 15-18 (emphasis added). Thus, the teaching of Example 2 is limited to this particular 21 bp fragment wherein the strands are covalently linked using an organic linker.

The disclosures of Examples 1 and 2 describe species of generic dsRNA size ranges disclosed in the priority documents, German Patent Application Nos. 199 03 713.2 (filed January 30, 1999) (Exhibit 2) and No. 199 56 568.6 (filed November 24, 1999) (Exhibit 3). In the '713.2 document, dsRNA of from "10-1000" bp, more preferably "250-250" bp were disclosed. The '568.6 introduced for the first time the ranges of "not more than 49" bp and "15-49" bp. These ranges therefore have effective priority dates of November 24, 1999. However, neither document explicitly disclosed a single dsRNA of "not more than 49" bp. The chemically-linked, duplex forming construct of 21 bp did not appear in the present application until the January 29, 2000, international filing.

There is no disclosure in either the International Application or either of the two priority documents of any rationale for selecting a dsRNA of a particular size. The written description is thus limited to ranges of between "10-1000" bp, "not more than 49" bp and "15-49" bp. There is no disclosure relating to subranges within the recited range. The only explicit examples are the 340 bp dsRNA of Example 1, and the 21 bp dsRNA of Example 2. However, these dsRNA molecules are distinct and do not support a subrange of 21-340 bp, because the 21 bp dsRNA further comprises an organic linker not disclosed for the dsRNA shown in Example 1. The specification of the '802 application contains no further teachings relating to the selection of subranges.

The disclosure of the '802 application thus provides no written description for any range of dsRNA molecule sizes other than "10-1000" bp, "not more than 49" bp, and "15-49" bp. Accordingly, the applicants are not entitled to anything other than these explicitly-recited ranges; anything more would contravene controlling legal precedent and the examination guidelines of the U.S. PTO. (*In re Lukach, 442 F.2d 967, 169 USPQ 795 (CCPA 1971)* (subgenus range was not supported by generic disclosure and specific example within the subgenus range); Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, P1, "Written Description" Requirement, 66 fed. Reg. 1099 (January 5, 2001)).

The claims are not enabled under 35 U.S.C. § 112, first paragraph

The dsRNA constructs disclosed in USSN 09/889,802 are not enabled throughout their full scope.

While the scope of the disclosure is facially broad, the disclosure's teachings do not enable one of ordinary skill in the art to practice the alleged invention without undue experimentation. In particular,

claims encompassing or reciting dsRNA constructs of "not more than 49" bp are not enabled, with the sole exception being claims limited to the single disclosed dsRNA construct of Example 2.

Example 2 describes inhibition of expression of the YFP gene in a murine fibroblast cell line NIH3T3. A plasmid carrying the YFP gene was microinjected into the cells together with one of two different dsRNAs targeted to the gene. The cells were then under the fluorescence microscope 3 hours after injection to detect (or not) any green-yellow fluorescence resulting from expression of the YFP protein. One of the molecules (designated dsRNA-YFP) was a fully double-stranded 315 bp-long RNA homologous to the 5'-end of the YFP gene. Another molecule (designated L-dsRNA), also targeting the YFP gene, had the chemical structure:

5'-ucgagcuggacggcgacguaa-C18-uuacgucgccguccagcucga-3'

wherein "C18" is a C18 linker. Under suitable conditions the 3'-most RNA sequence (uuacgucgccguccagcucga) hybridized to the 5'-most sequence (ucgagcuggacggcgacguaa) by Watson-Crick base pairing to form a 21 base pair duplex stabilized by the C18 linker (the specification does not describe how to make the C-18 linker).

The application reports that the L-dsRNA construct inhibited protein expression, thereby enabling this single construct *in vitro*. The application fails to disclose any other RNA duplex-forming constructs of "not more than 49" bp. To the extent the application enables dsRNAs of "not more than 49" bp, however, such enablement is limited to constructs stabilized by a chemical linker. For as the application states, "This result demonstrates that even shorter dsRNAs can be used for specifically inhibiting gene expression in mammals when the double strands are stabilized by chemically linking the single strands." P. 19, ll. 15-18 (emphasis added). Thus, the application not only fails to provide any teachings enabling dsRNA without a chemical linker, it actively teaches away from such dsRNA.

Yet even to the extent the application enables chemically linked dsRNA of "not more than 49" bp, substantial portions of the disclosed scope have been reported to be ineffective for the disclosed use of inhibiting expression of a target gene. For example, Czauderna *et al.*, *Nucleic Acids Research* **31**, 2705 (2003) (Exhibit 4), studied RNAi in mammalian (HeLa) cells and reported that as compared to a 19-mer dsRNA, a 17 base pair dsRNA showed "dramatically reduced [gene] silencing activity, suggesting that active siRNA duplexes must have a minimal length of (~19 nt), which is in a

agreement with experiments assessing activity of siRNA molecules with different duplex lengths in *Drosophila* extracts [Elbashir *et al.*, *EMBO J.* **20**, 6877 (2001) (Exhibit 5)]."

Elbashir *et al.*, *Genes Dev.* **15**, 188-200 (2001) (Exhibit 6), examined the precise length requirement of dsRNA for target RNA degradation under optimized conditions in Drosophila lysate. Three series of dsRNAs directed against firefly luciferase (*Pp*-luc) reporter RNA were synthesized. "Specific inhibition of target RNA expression was detected for dsRNAs as short as 38 bp, but dsRNAs having length 29-36 bp were not effective in this process. The effect was independent of the target position ...." *Id.* at p. 189.

In view of the foregoing, therefore, claims encompassing dsRNA having fewer than 19 base pairs or between 29-36 base pairs fail to satisfy 35 U.S.C. § 112, first paragraph, as the application fails to enable one of ordinary skill in the art to use such inoperative constructs for the disclosed purpose of inhibiting target gene expression. As dsRNA constructs comprising fewer than 19 base pairs or 29-36 base pairs are not enabled, claims to compositions comprising such constructs and methods of using such constructs to inhibit gene expression are not enabled either. And furthermore, the data presented in the specification are limited to cell culture systems – the application fails to enable *in vivo* utility.

The claims are anticipated and/or obvious

As discussed above, written description support for the constructs disclosed in the present application is limited and various embodiments have differing effective filing dates:

(a) dsRNA of 10-1000 bp (effective filing date = January 30, 1999), and

(b) dsRNA of fewer than 50 bp and dsRNA of 15-49 bp (effective filing date = November 24, 1999).

Based on these effective filing dates, the prior art described below render claims reciting the foregoing embodiments unpatentable as anticipated and/or obvious

Fire *et al.*, WO 99/32619 (published July 1, 1999) (Exhibit 7)

Fire *et al.* relates to methods and reagents for inhibiting a target gene in a eukaryotic, vertebrate, or human cell (page 9, lines 3-5 and 20-21) using dsRNA. Fire *et al.* teaches that the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a

portion of the target gene of interest. The reference teaches the introduction of RNA with partial or fully double-stranded structure into the cell or the intracellular environment (page 6, lines 2-4). The double-stranded structure may be formed by two complementary RNA strands (page 6, lines 20-22 and page 11, lines 6-7). The dsRNA contains a nucleotide sequence that is identical to a portion of the target gene (page 6, lines 24-26 and page 11, lines 14-15). The length of the identical nucleotide sequences can be at least 25 bases (page 11, lines 27-28).

In view of the above-described teachings, any claims reciting a dsRNA for inhibiting target gene expression comprising "not more than 49" bp or "15-49" bp, are clearly anticipated and/or rendered obvious by Fire *et al.*

Graham *et al.*, WO 99/49029 (PCT/AU99/00195) (published September 30, 1999) (Exhibit 8)

Graham *et al.* discloses methods and means for modulating, including reducing or inactivating (page 16, lines 20-21 and 26-29; page 17, lines 5-7 and 9-14), the expression of a target gene in a cell, tissue or organ using a double-stranded RNA. For example, the methods described in Graham *et al.* teach introducing into a cell or tissue one or more nucleic acid molecules comprising multiple copies of a nucleotide sequence that is either substantially identical or complementary to the sequence of a target gene or a region thereof (page 6, lines 21-30; page 8, line 26 to page 9, line 3; page 15, lines 4-11; page 22, lines 14-18). In one embodiment, Graham *et al.* teaches two or more copies (units) of the target gene presented in the opposite orientations on the same nucleic acid molecule, i.e., vector, (page 15, lines 13-14; page 33, lines 11-29; page 34, lines 1-12; page 35, lines 20-25). The resulting molecule transcribed from such vector would form a dsRNA wherein one strand of the dsRNA has a region which is complementary to the target gene. In addition, Graham *et al.* provides several vectors, such as those shown in Figures 24, 25, and 38, that can be used to form dsRNA from separate RNA single strands in a mammalian cell to specifically inhibit the expression of a target gene.

Graham *et al.* further teaches that the nucleic acid molecule comprises a nucleotide sequence having greater than 85% identity to 100% identity with the target sequence (page 8, lines 8-13; page 21, lines 23-28). The nucleotide sequence of each unit in the tandem or direct repeated sequence can comprise at least about 20-100 nucleotides in length (page 8, line 21). The dsRNAs disclosed in Graham *et al.* are formed either from separate RNAs or are connected at the ends by a "stuffer fragment" to form a hairpin structure; the stuffer fragment comprises nucleotides, amino acids,

carbohydrates, oligosaccharides, carbon atoms, analogs, homologs or derivatives thereof (page 15, lines 15-20). In one embodiment, the stuffer fragment can comprise a nucleotide sequence of at least about 10-50 nucleotides (page 15, lines 25-26).

The cell in which expression of the target gene is modified can be a prokaryotic or eukaryotic cell (page 8, lines 26-28). For example, Graham specifies that the cell can be any cell which is derived from a multicellular plant or animal, including cell and tissue cultures thereof (page 7, lines 15-20; page 15, lines 3-4; page 19, lines 19-28).

In view of the above-described teachings, any claims reciting a dsRNA for inhibiting target gene expression comprising "no more than 49" bp or "15-49" bp are clearly anticipated and/or rendered obvious by Graham *et al.* Furthermore, claims directed to dsRNAs with a chemical linker are also clearly anticipated by Graham *et al.*

Conclusion

The specification contains written description support for a very limited set of genera and species:

(a) dsRNA of "10-1000" bp (effective filing date = January 30, 1999),

(b) dsRNA of "not more than 49" bp and dsRNA of "15-49" bp (effective filing date = November 24, 1999), and

(c) a single chemically-linked, duplex-forming construct of 21 bp (effective filing date = January 29, 2000).

However, the genera in (a) and (b) are not enabled for their full breadth as significant portions (dsRNA of fewer than 19 bp and dsRNA with 29-36 bp) are inoperative for their disclosed use and, hence, non-enabled. And with regard to (c), the specification provides enablement and written description support for only the specific chemically-linked, duplex-forming 21 bp construct. But even to the extent that other constructs having "not more than 49" bp are enabled, they are limited by the applicants own word to those in which the RNA strands are stabilized by a chemical linker.

Furthermore, because the constructs in (b), above, have an effective filing date of November 24, 1999, the prior art discussed above anticipates and/or renders obvious claims to such constructs.

Service on Applicant

The undersigned does not know with certainty the name of the applicants or the name of their attorney. However, the published PCT application lists Roland Kreutzer and Stephan Limmer, both of

Germany, as applicants/inventors. Accordingly, a copy of this document (with attachments) is being served on each of them. Furthermore, the corresponding European application lists Ribopharma AG as the patent owner, and Ribopharma AG merged with Alnylam Pharmaceuticals, Inc., to form Alnylam Holding Co. On information and belief, the patent attorney representing Alnylam Holding Co. is Kathleen Williams. Accordingly, a copy of this document (with attachments) is being served on Ms. Williams. In addition, a copy of this document (with attachments) is being served on Alnylam Pharmaceuticals, who is believed to be the owner of this application. In case the foregoing information is incorrect, the undersigned is also filing a duplicate copy of this document (with attachments) with the PTO pursuant to 37 C.F.R. 1.291.

Date: Mar 4, 2004 By: Eric P Schellin

Eric Schellin